

### CLAIMS

1. Composition for the detection and early differentiated count of Gram-negative microscopic organisms, wherein it contains a mixture of substances of protein origin with a total nitrogen content from 9 to 20 %, being in the mixture in a relationship between 2: 1 to 24: 1 in respect to the content of the inhibitors for Gram-positive organisms, and also containing a mixture of organic and inorganic substances, being this mixture in a relationship between 0.5: 1 to 2: 1 to the mixture of substances of protein origin.
2. Composition according to claim 1, wherein the mixture of substances of protein origin contains pancreatic or papainic beef heart hydrolysate, enzymatic hydrolysate of milk proteins, microbial origin autolysates or hydrolysates and mixture of egg yolk proteins.
3. Composition according to claim 2, wherein the mixture of the substances of protein origin contains the pancreatic or papainic beef heart hydrolysate at a concentration between 25 and 75 % of said mixture.
4. Composition according to claim 2, wherein the mixture of the substances of protein origin contains the enzymatic hydrolysate of milk proteins at a concentration up to 15 % of said mixture.
5. Composition according to claim 2 wherein the mixture of the substances of protein origin contains the microbial origin autolysate or hydrolysate at a concentration between 15 and 25 % of said mixture.
6. Composition according to claim 2, wherein the mixture of the substances of protein origin contains the egg yolk proteins at a concentration up to 45 % of said mixture.
7. Composition according to claim 1, wherein the growth inhibitory substances for Gram-positive organisms are preferably cholic and deoxycholic acids and bile salts.
8. Composition according the claim 1, wherein the mixture of organic and inorganic substances comprises oxides of bivalent metals and siliceous compounds; pH indicators; alcohols that can be metabolized by enzymes of at least one of the organisms to identify; chromogenic compounds that can be split by enzymes of at least one of the organisms to identify; and growth promoting substances for Gram-negative organisms.
9. Composition according to claim 8 wherein, within the mixture of organic and inorganic substances, the oxides of bivalent metals and siliceous compounds are preferably  $3\text{MgO} \times 4\text{SiO}_2 \times \text{H}_2\text{O}$  and  $\text{SiO}_2 \times \text{H}_2\text{O}$ , which are used at a concentration from 6 to 32 % respecting to the total mass of the mixture.

10. Composition according to claim 4 wherein, within the mixture of organic and inorganic substances, the pH indicators are preferably phenol red and neutral red, which are used at a concentration from 0.03 to 0.18 % respecting to the total mass of the mixture.
- 5 11. Composition according to claim 8 wherein, within the mixture of organic and inorganic substances, the alcohol that can be metabolized by enzymes of at least one of the organisms to identify is preferably  $C_3H_8O_2$ , which is used in amounts from 10 to 14 mL/ L.
12. Composition according to claim 8 wherein, within the mixture of organic and inorganic compounds, the chromogenic compound that can be split by the action of enzymes of at least one of the organisms to identify is preferably X-gal, which is used at a concentration from
- 10 0,15 to 0.3 % respecting to the total mass of the mixture.
13. Composition according to claim 8 wherein, within the mixture of organic and inorganic compounds, the growth promoting substances for Gram-negative organisms are preferably sodium and magnesium salts, nitrogen compounds of low molecular weight and sulfured amino acids.
- 15 14. Composition according to claim 13, wherein the sodium and magnesium salts are preferably magnesium chloride and sodium carbonate, which are at concentrations from 0.03 to 32 % respecting to the total mass of the mixture.
- 20 15. Composition according to claim 13, wherein the nitrogen compound of low molecular weights is preferably creatinine, which is used at a concentration up to 3 % respecting to the total mass of the mixture.
16. Composition according to the claim 13, wherein the sulfured amino acids are preferably cystine and cysteine, which are used at concentrations up to 1.25 % respecting to the total mass of the mixture.
- 25 17. Composition according to claim 1, wherein within the mixture of organic and inorganic substances are included gelling agents, preferably agar with a hardness between 400 and 700 g/ cm<sup>2</sup>, which is used at a concentration from 40 to 63 % respecting to the total mass of the mixture.
18. Composition according to claim 1, wherein said composition has a pH value between 6,8 and 7,4.
- 30 19. Method for preparation the composition of claims 1 to 18, wherein said composition is prepared by suspending from 30 to 32 g of the composition in 1 L of a mixture of distilled or deionized water and alcohol, heating and cooling until jellification, inoculating the sample and incubation at temperature from 30 to 45 °C for 12 to 24 hours.

20. Method for the early detection and differentiated count of Gram negative microscopic organisms wherein the detection and count of *E. coli* and other coliform organisms is possible by the blue-greenish color of the colonies on the orange bottom of the medium; *Salmonella* non *typhi* for the red color of the center of the colonies on rosy bottom of the medium;

5 *Salmonella typhi* and *Proteus* for the transparency of the colonies; *Citrobacter* and *Klebsiella* for the violet color of the colonies on the pink orange bottom of the medium and *Pseudomonas aeruginosa* for the orange color with darker center of the colony, taking greenish pigmentation starting from 24 hours and producing greenish fluorescence under low ultraviolet light.